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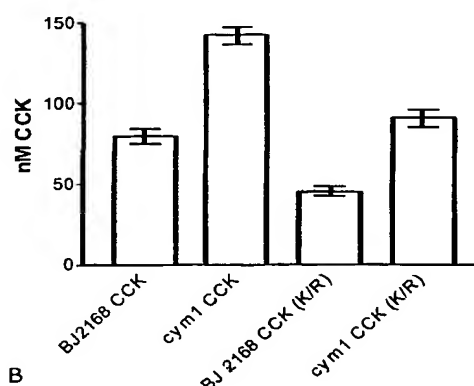
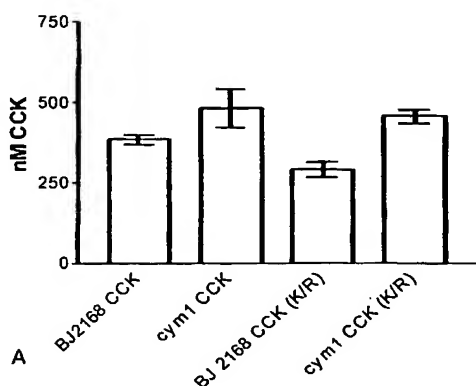
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR INCREASING THE PRODUCTION OF A RECOMBINANT POLYPEPTIDE FROM A HOST CELL



(57) Abstract: The present invention provides a host cell comprising a nucleic acid sequence encoding a recombinant polypeptide in which the production of a naturally occurring metalloprotease comprising the sequence HXXEH has been reduced or inhibited by genetic manipulation. The present invention also relates to methods for enhancing the production of a polypeptide from a cell by disrupting the synthesis or activity of the metalloprotease. In particular, the present invention relates to methods for enhancing the secretion of recombinant polypeptides from host cells such as, but not limited to, yeast and bacterial cells. The metalloprotease is a member of the ptillysin subfamily of proteases, characterized by comprising the sequence HXXEH, where X is any amino acid.

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/DK 03/00609

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 C12N15/57 C12P21/02 C12N1/19

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12P C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, SEQUENCE SEARCH

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 33960 A (ESTELL DAVID A ;GENENCOR INT (US)) 8 July 1999 (1999-07-08) claims 1-8 page 2, line 18 - line 26	1-22
X	WO 97 46689 A (GIST BROCADES BV ;DEN HOMBERGH JOHANNES PETRUS T (NL); VISSER JACO) 11 December 1997 (1997-12-11) cited in the application claims page 15, paragraph 5.4	1-22
X	WO 96 29391 A (NOVONORDISK AS ;LEHMBECK JAN (DK)) 26 September 1996 (1996-09-26) claims ---	1-22
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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Date of the actual completion of the international search

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International Application No

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>EGEL-MITANI M ET AL: "Yield improvement of heterologous peptides expressed in yps1-disrupted <i>Saccharomyces cerevisiae</i> strains"</p> <p>ENZYME AND MICROBIAL TECHNOLOGY, vol. 26, no. 9-10, June 2000 (2000-06), pages 671-677, XP002283345</p> <p>ISSN: 0141-0229</p> <p>abstract</p> <p style="text-align: center;">-----</p>	1-22

# INTERNATIONAL SEARCH REPORT

International Application No

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9933960	A	08-07-1999	AU 1832799 A	19-07-1999
			EP 1042487 A2	11-10-2000
			WO 9933960 A2	08-07-1999
			US 2003045438 A1	06-03-2003
			US 6465186 B1	15-10-2002
-----				
WO 9746689	A	11-12-1997	AU 3256397 A	05-01-1998
			WO 9746689 A1	11-12-1997
			EP 0907744 A1	14-04-1999
			JP 2000511429 T	05-09-2000
-----				
WO 9629391	A	26-09-1996	AU 4940096 A	08-10-1996
			WO 9629391 A1	26-09-1996
			EP 0815200 A1	07-01-1998
			JP 11502111 T	23-02-1999
			US 5968774 A	19-10-1999
			US 5861280 A	19-01-1999
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